FOXL2 AS GENETIC SEX MARKER ON WRECKFISH P*olyprion americanus*) BROODSTOCKS AND WILD CAUGHTS

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Introduction

Wreckfish (*Polyprion americanus*) is a species with an increasing interest in European aquaculture, for its easy adaptation to captivity, their rapid growth rate in the first years of life, and a high price in the market. However, this species has a very late sexual maturation and no apparent dimorphisms between males and females, which difficult the management of the broodstocks. In order to develop a genetic tool that allows identification of sex in this species, this study analyzes the levels of expression of FOXL2 gene, involved in ovarian differentiation in vertebrates, in different individuals with varying degrees of gonadal maturation, for assess its usefulness of this gene as a sex marker.

Material and Methods

Samples of gonadal and gill tissues were analyzed, obtained from wreckfish of different broodstocks established in Galicia (Spain) and wild specimens landed at fish markets. The set of samples covering the different stages of gonadal maturation, from immature juveniles to completely developed animals in their spawning period.

The samples were stored in RNAlater, and then the RNA were extracted with Trizol and purified with chloroform and isopropanol-ethanol washings. For expression analysis RT- qPCR was performed with the kit Brilliant III Ultra-Fast SYBR Green QPCR Master Mix Agilent Technologies, using primers specific for wreckfish FOXL2 developed by Mejuto et al. 2012 The experimental gene expression design was based on comparative analysis of the cycle value threshold (Ct), using B-actin as the endogenous control. These analyzes were performed in a One-Step Real-Time PCR System from Applied Biosystems.

Results and Discussion

The extraction and purification of RNA from both sample types (from dead specimens at fish markets and the living breeders) was achieved successfully, indicating the suitability of the method for processing of biological material. Preliminary results that the gene FOXL2 is already expressed in juveniles females in which no external signs of sexual maturation are appreciated (Ct mean= 27,66±0,6), and where the dissection of the gonads (in the case of dead specimens) is necessary for sex determination. This early expression of FOXL2 has already been observed in other teleost such as Nile tilapia (Wang et al., 2004). Its presence in wreckfish could be useful as a genetic tool to differentiate males from females in aquaculture stocks, especially given that this species reaches sexual maturity around 7-8 years of life (Peleteiro and Bruzón 2014), and whose only external difference between the sexes is a small variation in the structure of gonopore appreciable only during the high accretion gonad phases in each breeding season (Peleteiro pers. com.).

It is necessary to continue these expression analysis in order to consolidate this gene as a sex marker, and to assess whether expression levels in females can be used to infer what is their state of gonadal development. If so, would entail a less traumatic substitute for the animal of the gonadal biopsy techniques by cannulation used for this purpose, resulting in lower handling of animals and a greater welfare in their culture conditions.

References

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